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RELATIONSHIP BETWEEN SLUDGE VOLUME INDEX AND BIOMASS STRUCTURE WITHIN ACTIVATED SLUDGE SYSTEMS

D. P. Mesquita[1]; O. Dias[1]; A. L. Amaral[2]; E. C. Ferreira[1]

[1] IBB - Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal
Phone: (+351) 253 604 407 – Fax: (+351) 253 678 986 – Email: ecferreira@deb.uminho.pt
[2] Instituto Superior de Engenharia de Coimbra, Instituto Politécnico de Coimbra, Rua Pedro Nunes, Quinta da Nora, 3030-199 Coimbra, Portugal

ABSTRACT – In activated sludge systems, the sludge settling ability is considered a critical step where filamentous bulking and biomass deflocculation are the most common problems, causing the reduction of the effluent quality. Furthermore, in recent years image processing techniques have been successfully used to elucidate the activated sludge morphology. Keeping that in mind, a program was developed for the characterization of activated sludge collected from eight wastewater treatment plants comprising both good and poor settling sludge. The results showed a strong correlation between the sludge volume index, and image analysis based parameters emerging from filamentous and aggregated biomass contents, explaining the state of biological systems.

KEYWORDS: activated sludge, image analysis, aggregates, filaments, settling ability.

1. INTRODUCTION

Activated sludge systems are biological processes highly used in wastewater treatment plants (WWTP) relying on aggregated biomass in which the physical properties are important to guarantee an efficient pollution removal and sludge settling ability. However, when the operating conditions are not perfect mainly in terms of organic load, nutrients and oxygen contents some malfunctions may occur. The most common problems previously reported are: pinpoint bulking due to the absence of filamentous bacteria, leading to hardly settleable small flocs; filamentous bulking due to filamentous bacteria surplus, leading to hardly settleable linked flocs; dispersed growth due to bacteria not flocculating leading to non-settling turbid effluents; and zoogleal or viscous bulking due to large flocs with poor settling abilities and compaction leading to highly viscous and organic contents final effluents. In order to determine sludge settling ability, SVI (sludge volume index) is considered one of the most suited and widely used parameter. This critical factor, regulating the solid-liquid separation in biological systems has been increasingly related to the floc structure in activated sludge systems (Palm *et al*., 1980; Dagot *et al.*, 2001), especially on filamentous bulking surveys (Lee *et al.*, 1983).

In recent years, image processing and analysis methodologies have been increasingly used for activated sludge characterization. Sezgin (1982) established that the SVI is strongly influenced by floc size and filamentous bacteria contents. Other studies such as Matsui and Yamamoto (1984), Ganczarczyk (1994) and Grijspeerdt and Verstraete (1997) used automated image analysis to relate the microorganism's morphology in biological systems with the sludge settling properties. Recently, image analysis has been the basis of the assessment of biomass morphology changes using both bright field and phase contrast microscopy (da Motta *et al.*, 2001; Amaral and Ferreira, 2005; Jenné *et al.*, 2007). The combination between settling properties and image analysis parameters, such as the filamentous bacteria contents associated to flocs morphological characterization and biomass contents, may offer powerful information, and an immediate intervention can be made to the system. A strong relationship between the filamentous bacteria per biomass contents and SVI has been reported by Amaral and Ferreira (2005) in a filamentous bulking system, pointing towards the possibility of predicting bulking phenomena of filamentous nature by image analysis.

However, care should be taken with respect to the analysis of the obtained data in order to prevent image analysis methodologies faults as pointed out in the studies of da Motta *et al*. (2002), Martins *et al.* (2004) and Schuler and Jassby (2007). Keeping this in mind, in the present work eight wastewater treatment plants, with different operating conditions, were surveyed in order to establish a robust correlation between the sludge volume index and image analysis parameters.

2. MATERIAL AND METHODS

The activated sludge samples were collected from the aeration basins of eight WWTP, treating domestic effluents. For each sample, total suspended solids (TSS) were determined by weight, and later used to determine the SVI (APHA *et al.*, 1989). The biomass settling ability was measured in a cylindrical column (10 L) for 30 min. Furthermore, previous results (Amaral and Ferreira, 2005) were also included representing an earlier period of filamentous bulking conditions in one of the studied WWTP.

2.1 Image Acquisition

Around 200 images were acquired in bright field microscopy to obtain representative information about the sludge content. All the images in bright field were acquired in a *Leitz Laborlux S* optic microscope (*Leitz*, Wetzlar), with 100x magnification, coupled to a *Zeiss AxioCam* (*Zeiss*, Oberkochen). The images acquisition was performed in 1300 x 1030 pixels and 8-bit format through the commercial software *Axio Vision 3.1* (*Zeiss*, Oberkochen). The image acquisition of the previous survey relied on 20 images per sample acquired in bright field microscopy in a SZ 4045TR-CTV Olympus stereo microscope (Olympus, Tokyo), with 40x magnification, coupled to a Sony CCD AVC–D5CE (Sony, Tokyo) grey scale video camera. The images were acquired in 768×576 pixels and 8-bit format by a Data Translation DT 3155 (Data Translation, Marlboro) frame grabber using the commercial software package Image Pro Plus (Media Cybernetics, Silver Spring) (Amaral and Ferreira, 2005).

2.2 Image Processing

The image processing and analysis program for the aggregates and filaments characterization, was developed in *Matlab 7.3* (*The Mathworks, Inc.*, Natick) language, adapting a previous version developed by Amaral and Ferreira (2005). The image processing step determines the binary images from the aggregated biomass and the protruding filamentous bacteria later used in the image analysis step to determine the morphological parameters. Figure 1 shows a schematic representation of the main steps of the program, comprising the image pre-treatment, segmentation, and debris elimination whereas the image analysis program is oriented to the aggregates and filaments contents determination. A more detailed description about the program can be found in (Amaral and Ferreira, 2005).

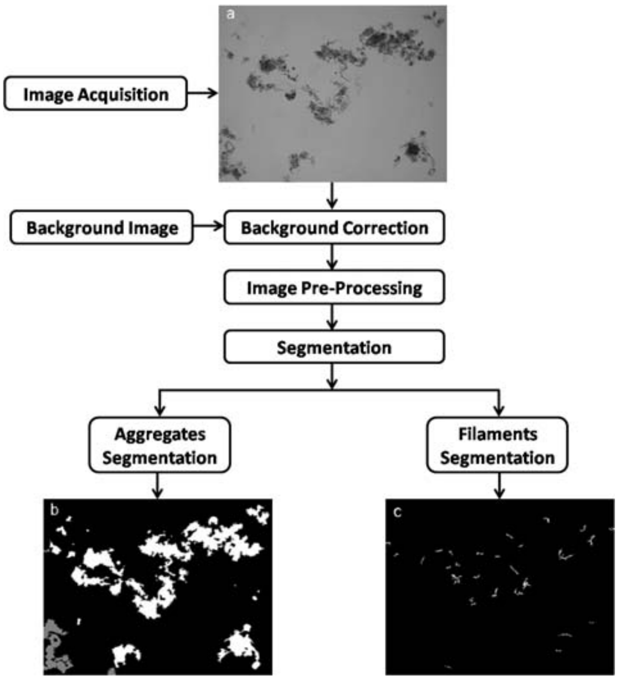

Figure 1 – Schematic representation of the image analysis program with the original image from the activated sludge system with 100x magnification (a), binary aggregates image (b), binary filaments image (c).

2.3 Image Analysis Parameters

Supported on the previous study of Amaral and Ferreira (2005) 5 parameters were determined either directly from the image analysis program either in association with the sludge physical properties for a total of 149 different samples (encompassing 400 000 aggregates). Total aggregates area (TA), total filaments length (TL), total filaments length per volume (TL/Vol), and total filaments length per total aggregated area ratio (TL/TA) were determined alongside the total filaments length per total suspended solids (TL/TSS) characterizing the aggregates and filaments dynamics. A more detailed description of each parameter can be found in Amaral and Ferreira (2005).

3. RESULTS AND DISCUSSION

Different studies have sought to determine the relationship between filament contents and sludge settling ability. Commonly, the sludge volume index (SVI) is the most suitable parameter to define the sludge settling ability. In the course of this survey, it was studied a wide range of SVI data, comprising both good and poor sludge settling ability properties. Table 1 presents the values range of TSS and SVI parameters for each WWTP.

Table 1 – TSS and SVI values for each WWTP studied.

WWTP	TSS (mg/L)	SVI (mL/g)
1	500 – 6500	150 – 260
2	1600 – 10500	60 – 200
3	3200 – 7700	100 – 270
4	900 – 6600	80 – 410
5	500 – 3100	20 – 100
6	200 – 3200	10 – 50
7	500 – 3200	40 – 120
8	900 – 4300	200 – 620

During this study, different approaches were performed based on previous researches (Palm *et al.*, 1980; da Motta *et al.*, 2001; Amaral and Ferreira, 2005; Jenné *et al.*, 2007). The first approach was to relate the SVI and the total filaments length per volume considering the six wastewater treatment plants as presented in Figure 2. The study of Palm *et al.* (1980) suggested the existence of a threshold value of 150 mL/g for SVI when the total filaments length per volume (TL/Vol) increased above 10 m/mL. That threshold value was considered the limit for bulking sludge presence in activated sludge systems.

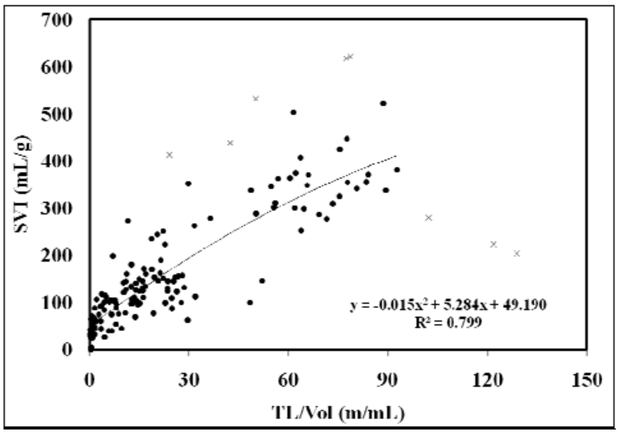

Figure 2 – Relationship between the SVI and filaments length (TL/Vol). Discarded outliers are represented by cross.

A PLS analysis was prior performed in order to determine possible outliers and discard them from the final analysis resulting in the identification of 8 outliers of a total of 149 samples (5.4%). The results herein reported, showed a poor 0.894 correlation coefficient (R^2 0.799) between the SVI and TL/Vol, even with the 8 identified outliers discarded. Furthermore, for the SVI values higher than 150 mL/g bulking limit, a large dispersion could be detected considering the different sludge samples. With respect to the study of Palm *et al.* (1980), the obtained results seem to point to a bulking limit around 20 m/mL, that is, twice as reported by these authors. Finally, when no filaments were present the SVI predicted by the TL/Vol analysis was around 50 mL/g (49.2 mL/g), which was further corroborated by the following analysis for the TL/TA and TL/TSS. Thus, this result seems to point to a minimum value for the SVI around 50 mL/g even when there are no visible bulking phenomena, although such assumption must be validated in subsequent studies for different aggregates structures.

As argued by Schuler and Jassby (2007), the filament contents per volume of sample are useful for comparing samples within a given study; however, comparing different plants can be problematic, since biomass concentrations changes between systems and over time, within the aerated basins. Hence, this parameter may not be the most suited to represent the sludge settling ability, due to reasons stated above.

Supported on the study of Amaral and Ferreira (2005), total filaments length per total aggregates area ratio (TL/TA) was also determined. These authors found this parameter to be correlated with the SVI providing valuable information about the biological system. Furthermore, a straight dependence of the SVI with the free filamentous bacteria per aggregates contents ratio could be considered for filamentous bulking phenomena. The relationship between the SVI and the free filamentous bacteria per aggregates contents is presented in Figure 3.

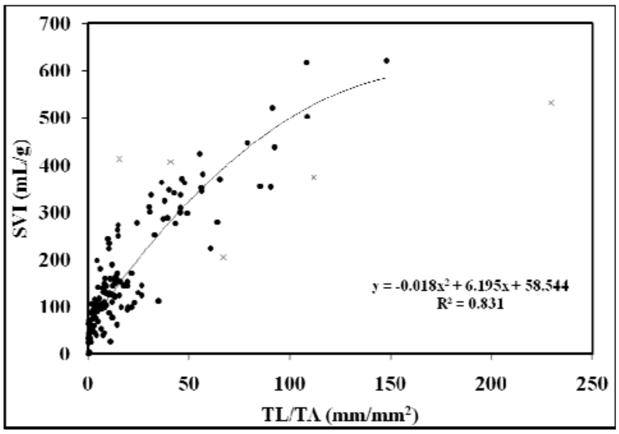

Figure 3 – Correlation between the SVI and TL/TA during the monitoring period. Discarded outliers are represented by cross.

As for the TL/Vol, a PLS analysis was prior performed in order to determine possible outliers and discard them from the final analysis resulting in the identification of 5 outliers of a total of 149 samples (3.4%). From Figure 3, a 0.912 correlation coefficient (R^2 0.831) was found between the TL/TA and the SVI values. As for the TL/Vol analysis, higher dispersion could be detected for the SVI values higher than the 150 mL/g bulking limit, considering the different sludge samples. Furthermore, still with respect to the bulking limit, it seems to correspond to a TL/TA around 15 mm/mm^2. As for the TL/Vol, when no filaments were present the SVI predicted by the TL/TA analysis was around 50 mL/g (58.5 mL/g), further corroborated by the subsequent analysis for the TL/TSS. Although still not fully satisfying, the current 0.912 correlation coefficient between SVI and TL/TA clearly surpasses the previous reported 0.839 correlation of Amaral and Ferreira (2005) study for a single wastewater treatment plant operating in filamentous bulking conditions (SVI values above 200 mL/g).

The study of Amaral and Ferreira (2005) introduced yet another parameter partially determined by image analysis and partially by physical measurements (TL/TSS) providing the best assessment of the SVI values. This parameter was, at the time, used to monitor the SVI behavior in a wastewater treatment plant operating in filamentous bulking conditions and, therefore, was also included in this study. Furthermore, according to Schuler and Jassby (2007), expressing filaments contents per mass is probably the most useful way for comparing filaments contents data from different studies and/or from samples with different biomass concentrations. Given the fact that such concentrations can vary greatly from one system to the other this approach normalizes filaments contents to biomass. As the SVI test is a settled volume normalized to biomass content, it is appropriate that filaments contents should also be normalized to biomass when comparing the two.

Thus, the correlation between SVI and TL/TSS was performed (Figure 4), since this parameter is probably the most useful way to compare filaments contents collected from either different aerated tanks or single systems oscillating significantly during the monitoring period.

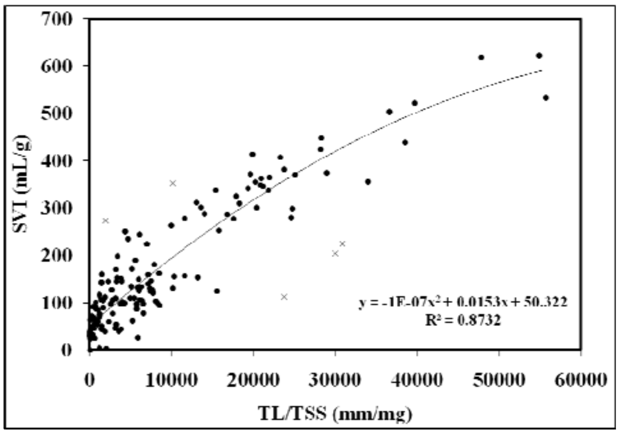

Figure 4 – Correlation between the SVI and TL/TSS during the monitoring period. Discarded outliers are represented by cross.

Once again, a PLS analysis was prior performed in order to determine possible outliers and discard them from the final analysis resulting in the identification of 5 outliers of a total of 149 samples (3.4%). Analyzing Figure 4 it could be withdrawn a satisfactory 0.934 correlation coefficient (R^2 0.873) between the TL/TSS and the SVI values. Contrary to the TL/Vol and TL/TA analyses, no significant increase on dispersion could be detected for the SVI values higher than the 150 mL/g bulking limit. With respect to the study of Sezgin (1982), the obtained results seem to point to a bulking limit around 7 000 mm/mg, that is, 30% below the limit reported by this author (10 000 mm/mg). Finally, and in accordance to the findings of both TL/Vol and TL/TA analysis, when no filaments were present the SVI predicted by the TL/TSS analysis was around 50 mL/g (50.3 mL/g), thus corroborating the previous findings. With respect to Amaral and Ferreira (2005) study for a single wastewater treatment plant operating in filamentous bulking conditions, the obtained 0.934 correlation coefficient clearly outperforms the previously reported value of 0.885.

In the course of this survey, it was possible to study a wider range of plant operating conditions, contrasting to Amaral and Ferreira (2005) study on filamentous bulking conditions. As a result of that, the attained correlations reflect a more complete and wider analysis of both non-bulking and filamentous bulking conditions.

4. CONCLUSIONS

The obtained results allowed explaining the strong relationships between sludge settling properties and free filamentous bacteria contents establishing relevant relationships between the macroscopic and microscopic properties of the biological system. This study also emphasized the advantages of combining SVI determination with filamentous bacteria based descriptors in order to establish the true nature of the phenomena occurring within the biological system. In conclusion, it was found that the proposed image analysis methodology has provided relevant information concerning the plants sludge settling ability complementing conventional survey methods.

5. ACKNOWLEDGMENTS

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6. REFERENCES

AMARAL, A.L.; FERREIRA, E.C. Activated sludge monitoring of a wastewater treatment plant using image analysis and partial least squares regression. *Anal. Chim. Acta*, 544, p. 246-253, 2005.

ANDREADAKIS, A.D. Physical and chemical properties of activated sludge floc. *Wat. Res.*, 27 p. 1707-1714, 1993.

APHA; AWWA; WPCF. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, Washington, DC, 1989.

da MOTTA, M.; AMARAL, A.L.; NEVES, L.; ARAYA-KOFF, P.; FERREIRA, E.C.; ALVES, M.M.; MOTA, M.; ROCHE, N.; VIVIER, H.; PONS, M.N. Efeito da Diluição na Caracterização da Biomassa de Sistemas de Tratamentos de Efluentes por Análise de Imagem. Proceedings of the 14th Brazilian Congress on Chemical Engineering, CD-ROM, 9 pp., 2002.

da MOTTA, M.; PONS, M.N.; ROCHE, N. Automated monitoring of activated sludge in a pilot plant using image analysis. *Wat. Sci. Tech.*, v. 43(7), p. 91-96, 2001.

DAGOT, C.; PONS, M.N.; CASELLAS, M.; GUIBAUD, G.; DOLLET, P.; BAUDU, M. Use of image analysis and rheological studies for the control of settleability of filamentous bacteria: application in SBR reactor. *Wat. Sci. Tech.* v. 43(3), p. 27-33, 2001.

GANCZARCZYK, JJ. Microbial Aggregates in Wastewater Treatment. *Wat. Sci Tech.*, v. 30, p. 87-95, 1994.

GRIJSPEERDT, K.; VERSTRAETE, W. Image analysis to estimate the settleability and concentration of activated sludge. *Wat. Res.*, v. 31, p. 1126-1134, 1997.

JENNÉ, R.; BANADDA, E.N.; SMETS, I.Y.; DEURINCK, J.; VAN IMPE, J.F. Detection of Filamentous Bulking Problems: Developing an Image Analysis System for Sludge Composition Monitoring. *Microscopy and Microanalysis*, v. 13, p. 36-41, 2007.

LEE, S.E.; KOOPMAN, B.; BODE, H.; JENKINS, D. Evaluation of alternative sludge settleability indexes. *Wat. Res.*, v. 17(10), p. 1421-1426, 1983.

MARTINS, A.M.P.; PAGILLA, K.R.; HEIJNEN, J.J.; VAN LOOSDRECHT, M.C.M. Bulking filamentous sludge - a critical review. *Wat. Res.*, v. 38(4), p. 793–817, 2004.

MATSUI, S.; YAMAMOTO, R. The use of a color TV technique for measuring filament length and investigating sludge bulking causes. *Wat. Sci. Tech.*, v. 16, p. 69-81, 1984.

PALM, J.C.; JENKINS, D.; PARKER, D.S. Relationship between organic loading, dissolved-oxygen concentration and sludge settleability in the completely-mixed activated-sludge process. *J. Water Pollut. Control Fed.* v. 52(10), p. 2484-2506, 1980.

SCHULER, A.; JASSLY, D. Filament content threshold for activated sludge bulking: Artifact or Reality? *Wat. Res.*, v. 41, p. 4349-4356, 2007.

SEZGIN, M. Variation of Sludge Volume Index with Activated Sludge Characteristics. *Wat. Res.*, v. 16, p. 83-88, 1982.